

Methods & Materials: Consecutive non-duplicate clinical isolates of *Pseudomonas aeruginosa* were collected from the patients admitted to surgical ward of Silchar Medical College and Hospital in the duration of September 2013 to August 2014. The isolates were screened for polymyxin resistance by Kirby-Bauer disc diffusion method and the minimum inhibitory concentrations. mRNA and cDNA of five selected polymyxin resistant strains representing different MIC range were isolated in normal condition of the strain as well as after treating with FeCl₃ alone and FeCl₃ and polymyxin antibiotic. Transcriptional expression was observed for *pmrB* and *arnA* by quantitative real time PCR in reference to *P. aeruginosa* PAO1. Susceptibility pattern of these polymyxin resistant strains was performed by Kirby-Bauer disc diffusion method. DNA fingerprinting of the isolates was carried out by performing REP PCR.

Results: A down regulated expression of *pmrB* and *arnA* was observed in polymyxin resistant strains of *P. aeruginosa* which is unique comparing to other studies. Low susceptibility rate to amikacin and gentamicin, β -lactam- β -lactamase inhibitor piperacillin-tazobactam and quinolone group of drug ciprofloxacin was shown by polymyxin resistant *P. aeruginosa* strains whereas total resistance was observed in case of third generation cephalosporin cefepime. REP PCR results showed these polymyxin resistant organisms are heterogenous by showing different clonal pattern.

Conclusion: This study highlights the urgency of obtaining knowledge on the pharmacology of polymyxins to optimize their clinical use and minimize potential for development of resistance. This will help in management of treatment and infection control due to multidrug resistant *P. aeruginosa*.

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Molecular epidemiology and spread dynamics of multi-drug resistant in *A. baumannii* isolated from patients and hospital environment in Bangladesh

R. Farzana^{1,*}, T. Mozumder², B. Hasan³

¹ Khwaja Yunus Ali Medical College, Enayetpur, Sirajgonj, Bangladesh

² Z.H Sikder Medical College, Dhaka, Bangladesh

³ Uppsala University, Sweden, Uppsala, Sweden

Background: Multidrug-resistant (MDR) *A. baumannii* has been a serious challenge in the hospitals globally including Bangladesh. The study was performed to investigate molecular epidemiology & spread dynamics of antibiotic resistant *A. baumannii* both from patients and hospital environment in Bangladesh.

Methods & Materials: A set of 49 clinical *Acinetobacter* strains were collected from five clinical microbiology labs located in Dhaka, Bangladesh during 2014–2015. Additionally, 100 samples were collected from different hospital surfaces of Dhaka Medical College Hospital. All strains and samples were cultured on CHROMagar™ *Acinetobacter* selective media. *A. baumannii* was

according to EUCAST and CLSI. PCR was used to detect different resistance genes; quinolones (*qnrS1*, *aac6*, *qepA*, *qnrC1*, *qnrB1*, *qnrA1*), 16S methylase (*rmtA*, *rmtB*, *rmtC*, *rmtD*, *armA*) and OXAs (-23, -24, -58, -143). Real-time multiplex PCR were conducted for the presence of carbapenem resistant genes (*NDM*, *VIM*, *IMP*, *KPC* and *oxa-48*). Epidemiological typing & clonal profile was performed by rep-PCR.

Results: 95% (47/49) human and 31% (10/32) environmental isolates of *A. baumannii* had growth on CHROMagar™ agar. All clinical and 10 environmental strains carried *bla*_{OXA-51} gene which confirmed as *A. baumannii*. Resistance to 4 or more antibiotic classes was found in 48 clinical and 10 environmental strains. Forty clinical and all environmental strains carried *bla*_{OXA-23} however; *bla*_{OXA-58} was in one clinical strain. The predominant ciprofloxacin resistant gene was *aac6* in both clinical and environmental isolates followed by *qnrB1* in clinical isolates and *qnrC1* in environmental isolate. Only *armA* gene was found in clinical and environmental strains. None of the clinical and environmental strains were positive for other carbapenem resistant genes (*NDM*, *VIM*, *IMP*, *KPC*, *OXa-48*). In total, 36 different clones were identified from both patients and environment; 6 different clinical clones (AC, BC, DC, FC, HC, PC) were common in different hospitals among patients. Some of the clones (CC, RC, P3, P6) were common both in patients and environmental strains.

Conclusion: The magnitude of resistance including their phenotypes and genotypes, and clonal relatedness among clinical and environmental *A. baumannii* indicates multi-drug resistant strains were wide spread in Bangladeshi hospitals.

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Genotyping of mycobacterium tuberculosis strains isolated from patients with pulmonary drug resistant tuberculosis in Ukraine

O. Konstantynovska¹, A. Rogozhin¹, A. Gerilovych^{2,*}, S. Sapko², P. Poteiko¹, O. Liashenko³, V. Bolotin², O. Solodianskin²

¹ Kharkov Medical Academy of Post-Graduate Education, Kharkiv, Ukraine

² National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine

³ V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

Background: The epidemiological situation of tuberculosis in Ukraine is extremely unfavorable – about 40 thousand people become sick on tuberculosis each year and about 10 thousand patients die. Changing of the contemporary socio-economic and environmental conditions plays an important role in the deterioration of the tuberculosis epidemic situation. Moreover drug resistance of *M. tuberculosis* is one of the main factors limiting the effectiveness of TB treatment. Due to the development of molecular genetics it has become possible to conduct genetic typing of



bacteria, which allows distinguishing between different strains of the pathogen isolated from TB patients.

Methods & Materials: During 2014 the 93 cases of TB were studied in patients who were treated in hospitals in Kharkiv region in Ukraine. Mycobacterium identification and testing of the drug susceptibility were performed as recommended by WHO. The samples of expectoration were used for the strain isolation on Lowenstein-Jensen medium. VNTR genotyping was done by using sets of primers for amplification of five exact tandem repeat ETR loci as previously described (Frothingham, R., 1998).

Results: It has been shown that 69% from isolated *M. tuberculosis* strains belong to Beijing family whereas 13% – to LAM. Other genotypes consisted 18% from all obtained isolates. The most common profile was 42435.

The most common resistance among Beijing strains were observed to streptomycin (100%), followed by resistance to isoniazid (99%), rifampicin (96%) and ethambutol (89%). Kanamycin and ofloxacin did not inhibit growth of *M. tuberculosis* isolates in 69% and 60% cases respectively. The most effective anti-TB drug was cycloserine (10%). Among LAM strains it was found resistance to kanamycin, isoniazid and streptomycin in 12 (100%) cases, ethambutol and rifampicin – in 11 (91.5%) cases.

The frequency of resistance to kanamycin, 4-Aminosalicylic acid and ethionamide was revealed significantly higher for LAM strain in compare to Beijing strain ($p < 0.05$). 41 (44%) of 93 patients were diagnosed as MDR TB and 52 (56%) – TB with extending resistance.

Conclusion: In conclusion, the data showed that TB in Kharkiv region is mostly characterized as multi-drug resistance cases. To prevent circulation and transmission of *M. tuberculosis* TB control program needs to be improved in Ukraine.

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Of bugs and drugs: have carbapenems met their doom?



M. Gupta^{1,*}, V. Agarwal², R. Surpam³

¹ SGPGI, Lucknow, Uttar pradesh, India

² Government Medical college, Nagpur, Nagpur, India

³ Government medical college, Nagpur, Nagpur, India

Background: Microorganisms though diminutive are formidable foes. The antibiotic soup that now permeates health-care facilities has exerted a selection pressure on pathogens and commensal organisms alike, and resistance has proliferated and spread such that many bacteria can withstand almost all drugs.

Detection of β -lactamases by virtue of their diverse complexity is a diagnostic dilemma hindered by the heterogeneity of both host and enzyme. Expression of multiple β -lactamases and their co-existence is a common parlance particularly in nosocomial pathogens which are notorious for disseminating them compromising the therapeutic alternatives. When faced with this mélange of enzymes in resource constraint settings lack or inadequacy of diagnostic tests may have grave implications.

Methods & Materials: 168 clinical isolates *E. coli* (n=53) *K. pneumoniae* (n=115) from invasive site infections were studied for their antimicrobial susceptibility and detection of β -lactamase

by inhibitor based disc potentiation test (DPT). A total of 18 agents of which 6 were substrate inhibitor combination discs were tested per isolate. Detection of ESBL, MBL, AmpC and KPC was done by using inhibitors- EDTA, Dipicolinic acid (DCA), Phenyl boronic acid (PBA), Clavulanic acid (CA) and cloxacillin (CLOX). Appropriate controls were incorporated.

Results: High resistance to all classes of cephalosporins was observed in all isolates. Carbapenem resistance was found in 87.15% isolates. Least resistance to cotrimoxazole and gentamicin was seen among the antimicrobials tested. ESBL was the most frequent β lactamase detected followed by MBL>AmpC>KPC.



KPC producer



KPC and MBL producer



Conclusion: We are living in a world where we will never be able to stay ahead of the bacterial mutation curve. A test of our resilience is how far behind the curve we allow ourselves to fall. Limited data is available on presence of KPC's in India and none from our region. There is a need for enhanced adherence with effective infection control measures, detect and protect strategy and antibiotic stewardship to curb patient-to-patient transmission and to reduce the selection of multidrug-resistant bacteria. Currently the main issues are providing practical recommendations on detection, treatment and prevention in different resource settings.

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